nucleic acid, the relative ability of an oligonucleotide to bind to the complementary nucleic acid may be compared by determining the melting temperature of a particular hybridization complex. The melting temperature (T_m), a characteristic physical property of double helices, denotes the temperature (in degrees centigrade) at which 50% helical (hybridized) versus coil (unhybridized) forms are present. T_m is measured by using the UV spectrum to determine the formation and breakdown (melting) of the hybridization complex. Base stacking, which occurs during hybridization, is accompanied by a reduction in UV absorption (hypochromicity). Consequently, a reduction in UV absorption indicates a higher T_m. The higher the T_m, the greater the strength of the bonds between the strands.

[0010] Oligonucleotides may also be of therapeutic value when they bind to non-nucleic acid biomolecules such as intracellular or extracellular polypeptides, proteins, or enzymes. Such oligonucleotides are often referred to as 'aptamers' and they typically bind to and interfere with the function of protein targets (Griffin, *et al.*, *Blood,* (1993), 81:3271-3276; Bock, *et al.*, *Nature*, (1992) 355: 564-566).

[0011] Oligonucleotides and their analogs have been developed and used for diagnostic purposes, therapeutic applications and as research reagents. For use as therapeutics, oligonucleotides must be transported across cell membranes or be taken up by cells, and appropriately hybridize to target DNA or RNA. These critical functions depend on the initial stability of the oligonucleotides toward nuclease degradation. A serious deficiency of unmodified oligonucleotides which affects their hybridization potential with target DNA or RNA for therapeutic purposes is the enzymatic degradation of administered oligonucleotides by a variety of intracellular and extracellular

ubiquitous nucleolytic enzymes referred to as nucleases. For oligonucleotides to be useful as therapeutics or diagnostics, the oligonucleotides should demonstrate enhanced binding affinity to complementary target nucleic acids, and preferably be reasonably stable to nucleases and resist degradation. For a non-cellular use such as a research reagent, oligonucleotides need not necessarily possess nuclease stability.

[0012] A number of chemical modifications have been introduced into oligonucleotides to increase their binding affinity to target DNA or RNA and resist nuclease degradation.

[0013] Modifications have been made to the ribose phosphate backbone to increase the resistance to nucleases. These modifications include use of linkages such as methyl phosphonates, phosphorothioates and phosphorodithioates, and the use of modified sugar moieties such as 2'-O-alkyl ribose. Other oligonucleotide modifications include those made to modulate uptake and cellular distribution. A number of modifications that dramatically alter the nature of the internucleotide linkage have also been reported in the literature. These include non-phosphorus linkages, peptide nucleic acids (PNA's) and 2'-5' linkages. Another modification to oligonucleotides, usually for diagnostic and research applications, is labeling with non-isotopic labels, *e.g.*, fluorescein, biotin, digoxigenin, alkaline phosphatase, or other reporter molecules.

[0014] A variety of modified phosphorus-containing linkages have been studied as replacements for the natural, readily cleaved phosphodiester linkage in oligonucleotides. In general, most of them (such as the phosphorothioate, phosphoramidates, phosphonates and phosphorodithioates) result in oligonucleotides with reduced binding to complementary targets and decreased hybrid stability. At least one dozen phosphorothioate oligonucleotides and derivatives

are presently being used as antisense agents in human clinical trials for the treatment of various disease states. The antisense drug Vitravine™, for use to treat cytomegalovirus (CMV) retinitis in humans, has been approved by regulatory agencies and is comedically marketed.

[0015] The structure and stability of chemically modified nucleic acids is of great importance to the design of antisense oligonucleotides. Over the last ten years, a variety of synthetic modifications have been proposed to increase nuclease resistance, or to enhance the affinity of the antisense strand for its target mRNA (Crooke *et al., Med. Res. Rev.*, **1996**, *16*, 319-344; De Mesmaeker *et al., Acc. Chem. Res.*, **1995,** *28,* 366-374).

[0016] RNA exists in what has been termed "A Form" geometry, while DNA exists in "B Form" geometry. In general, RNA:RNA duplexes are more stable, or have higher melting temperatures (Tm) than DNA:DNA duplexes (Sanger *et al., Principles of Nucleic Acid Structure,* **1984**, Springer-Verlag; New York, NY.; Lesnik *et al., Biochemistry,* **1995,** *34,* 10807-10815; Conte *et al., Nucleic Acids Res.*, **1997,** *25,* 2627-2634). The increased stability of RNA has been attributed to several structural features, most notably the improved base stacking interactions that result from an A-form geometry (Searle *et al., Nucleic Acids Res.*, **1993**, *21*, 2051-2056). The presence of a hydroxyl group in the 2'-pentofuranosyl (*i.e.*, 2'-sugar) position in RNA is believed to bias the sugar toward a C3' *endo* pucker (also known as a Northern pucker), which causes the duplex to favor the A-form geometry. On the other hand, 2'-deoxy nucleic acids (those having 2'-deoxy-*erythro*-pentofuranosyl nucleotides) prefer a C2' *endo* sugar pucker (also known as Southern pucker), which is thought to impart a less stable B-form geometry (Sanger, W. (1984) *Principles of Nucleic Acid Structure*, Springer-Verlag, New York, NY). In addition, the 2' hydroxyl groups of RNA can form